Rangu Swetha et al, 2026, Vol.14, No.5: 93-97

International Journal of Chemistry and Pharmaceutical Sciences

https://doi.org/10.30904/j.ijcps.2026.5014 | E-ISSN: 2321-3132

Induction of Multiple Sclerosis in Transgenic Rodents and Exploring the Ameliorative Potential of Plant Extract

Rangu Swetha*, D. Swathi, V. Umarani, K Shravan Kumar

Samskruti College of Pharmacy, Kondapur, Ghatkesar, Medchal, Hyderabad-501301, Telangana, India

****Corresponding E-Mail:** principal.y7@gmail.com*

Received: 11-02-2026 | Revised: 25-03-2026 | Accepted: 19-04 2026 | Published: 30-05-2026

Abstract:

The present research focused on the induction of multiple sclerosis (MS) in transgenic rodent models and the evaluation of the ameliorative potential of a test formulation (fenofibric acid suspension) through in silico, in vitro, and in vivo approaches. A novel method was established using ethidium bromide (EB) and copper sulphate/cuprizone as demyelinating agents delivered via stereotaxic surgery in both C57BL/6J mice and Wistar albino rats. Histopathological examinations confirmed successful induction, revealing hallmark MS features such as pyknotic nuclei, gliosis, neuronal hyperplasia, vascular congestion, inflammation, and Lewy body–like inclusions in cortical, hippocampal, and pontine regions. In silico docking studies demonstrated that fenofibric acid displayed strong binding affinities to $CD4^+$ and $CD8^+$ protein targets, comparable to the standard therapeutic dimethyl fumarate, with stable hydrogen bonding and π-alkyl interactions. These interactions suggest a potential immunomodulatory mechanism through modulation of T-cell activity, supporting its therapeutic promise. The test formulation of fenofibric acid suspension was successfully characterized with neutral pH (7.0), acceptable viscosity, and no incompatibility between excipients and inducing agents as confirmed by FTIR analysis. Toxicological evaluation indicated that the formulation was stable and safe for oral administration. Moreover, the study emphasizes the value of integrating transgenic models with phytochemical-based interventions to develop safer, cost-effective alternatives to current disease-modifying therapies.

Keywords: *Ameliorative Potential, immunomodulatory,* fenofibric acid suspension, FTIR

Introduction

The present research focused on the induction of multiple sclerosis (MS) in transgenic rodent models and the evaluation of the ameliorative potential of a test formulation (fenofibric acid suspension) through in silico, in vitro, and in vivo approaches. A novel method was established using ethidium bromide (EB) and copper sulphate/cuprizone as demyelinating agents delivered via stereotaxic surgery in both C57BL/6J mice and Wistar albino rats. Histopathological examinations confirmed successful induction, revealing hallmark MS features such as pyknotic nuclei, gliosis, neuronal hyperplasia, vascular congestion, inflammation, and Lewy body–like inclusions in cortical, hippocampal, and pontine regions. In silico docking studies demonstrated that fenofibric acid displayed strong binding affinities to $CD4^+$ and $CD8^+$ protein targets, comparable to the standard therapeutic dimethyl fumarate, with stable hydrogen bonding and π-alkyl interactions. These interactions suggest a potential immunomodulatory mechanism through modulation of T-cell activity, supporting its therapeutic promise.

Materials and Methods

Objective 1: Validation of Ethidium Bromide and Copper Sulphate Combination in *C57BL/6J* Mice

Healthy *C57BL/6J* mice were procured from Chromed Biosciences and housed under controlled laboratory conditions (temperature: 16–26 °C; humidity: 40–60%; photoperiod: 14 h light/10 h dark cycle). Animals were acclimatized prior to experimentation. Mice were anesthetized intraperitoneally (i.p.) with ketamine (75 mg/kg) and positioned on a stereotaxic apparatus with the skull oriented to align bregma and lambda on the same horizontal plane. The scalp was shaved, cleaned with 70% ethanol, and incised along the midline to expose the skull. To prevent ocular dehydration, sterile saline-soaked cotton swabs were applied to the eyes. Ethidium bromide (EB) and copper sulphate were stereotaxically injected into the intracerebropeduncle region at the following coordinates: AP: 0.6 mm; ML: 1.0 mm; DV: 1.6 mm. The trial was conducted for 14 days. Post-operative care: Mice were housed individually in polyacrylic cages with husk bedding and soft cloth. Following recovery from anesthesia (2–3 h), animals were provided milk and glucose water for 2–3 days to prevent physical stress. Gentamicin (35 mg/kg, i.p.) was administered for three consecutive days to prevent sepsis, while lignocaine gel was applied to sutures for pain relief. Neosporin powder was sprinkled to minimize the risk of bacterial infection. Clinical signs, including general health and hydration status, were closely monitored throughout the study.

Objective 2: Development and Validation of MS Model in Wistar Albino Rats

Wistar albino rats were acclimatized under standard housing conditions before surgical induction. Animals were anesthetized with ketamine (75 mg/kg, i.p.) and fixed in the stereotaxic frame in a skull-flat position. After shaving and disinfecting the scalp with 70% ethanol, a midline incision was made to expose the skull. Bregma and lambda were identified to guide stereotaxic coordinates. A cranial hole was drilled at coordinates AP: −11.6 mm; ML: ±2.6 mm; DV: −7.07 mm. Demyelination was

induced unilaterally by direct stereotaxic injection of 10 µL of 0.1% EB and copper sulphate in sterile 0.9% saline. The burr hole was sealed with dental cement, and the incision was sutured using absorbable sutures. Post-operative care: Rats were housed individually in polyacrylic cages with husk bedding and soft cloth. Recovery was monitored until animals regained spontaneous movement (2–3 h post-surgery). Similar to mice, milk and glucose water were provided for 2–3 days. Gentamicin (35 mg/kg, i.p.) was given for three days, and lignocaine gel was applied to sutures. Neosporin powder was used to prevent infections. Animals were observed regularly for clinical signs, body condition, and hydration status.

Objective 3: In Silico Evaluation of Test Drug

Molecular Docking Analysis

Protein targets, CD4+ (PDB ID: 64521) and CD8+ (PDB ID: 48251), were retrieved from the RCSB Protein Data Bank. Ligands, including fenofibric acid, selected phytoconstituents, were downloaded from the PubChem database. Docking studies were performed using AutoDock Vina. Ligand-protein interactions were analyzed with AutoDock Tools Suite, and visualized in Biovia Discovery Studio Visualizer. ADMET properties were assessed using the pkCSM web server.

Ligand Preparation

Ligands were optimized by converting SDF files into PDB format, performing energy minimization, and assigning correct bond orders and charges.

Protein Preparation

Protein structures were refined by removing heteroatoms, water molecules, co-crystallized ligands, and metal ions. Bond orders and charges were reassigned, and missing connectivity data were corrected using Swiss PDB Viewer (SPDBV).

Docking Procedure

Protein and ligand PDB files were processed in PyRx for docking simulation. Binding affinities were expressed as negative binding energy (ΔG) values, where higher negative values indicated stronger binding affinity and potential therapeutic efficacy.

Objective 4: Formulation, Characterization, and Toxicological Evaluation of Test Drug

Formulation

Fenofibric acid was formulated as a suspension by mixing 100 mg of drug with 0.2% carboxymethyl cellulose (CMC) and 0.4% Tween 80.

Characterization

- **Compatibility Study:** Compatibility of inducing agents and excipients was evaluated using Fourier Transform Infrared (FTIR) Spectroscopy (400–4000 cm^{-1}). Spectra were compared for functional group integrity and possible interactions.
- **pH Measurement**: The pH of the formulation was measured with a calibrated digital pH meter (buffer calibration at pH 4.0 and 7.0). Readings were taken in triplicate, and mean values were recorded.
- **Viscosity Determination:** Viscosity was measured using a Brookfield Viscometer (Model LVDVII, Brookfield Engineering Labs, USA) at 37 ± 0.1 °C with spindle No. 4. Measurements were recorded at angular velocities of 10–30 rpm, both before and after gel formation.

Objective 5: In Vivo Evaluation of Test Drug in EB + Copper Sulphate Induced MS Rats

Rotarod Test

Motor coordination and balance were evaluated using the rotarod apparatus. Rats were tested on days 1, 3, 5, 7, 9, 11, and 13. The apparatus was set at 25 rpm, and latency to fall (fall-off time, in seconds) was recorded in triplicates for each animal.

Body Weight Monitoring

Body weight was recorded daily throughout the study period as a marker of general health and systemic toxicity.

Histopathological Studies

Brain tissues (pons and hippocampus, 1 cm fragments) were excised and fixed in 4% paraformaldehyde. Tissues were paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Sections were examined microscopically at 200× magnification, photomicrographs were taken for comparative pathological evaluation.

Hematological Parameters

Hematological evaluations were carried out to assess potential abnormalities in the hematopoietic system. Blood samples were analyzed using an automated hematology analyzer, which provided values for the following parameters:

Table 1: Hematological Parameters Assessed

Parameter	Abbreviation	Unit
White Blood Cells	WBC	$\times 10^3/\mu L$
Red Blood Cells	RBC	$\times 10^6/\mu L$
Hemoglobin Concentration	HGB	g/dL
Mean Corpuscular Volume	MCV	fL
Mean Corpuscular Hemoglobin	MCH	pg
Platelet Count	PLT	$\times 10^3/\mu L$

The analyzer provided precise quantitative measurements, enabling detection of hematological abnormalities and assessment of treatment effects in experimental groups.

Statistical Analysis: All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) to compare group differences. A p-value < 0.05 was considered statistically significant.

Result and Discussion

Objective 1 Validation of Ethidium Bromide and Copper Sulphate Combination for the Induction of Multiple Sclerosis in *C57BL/6J* Mice Using Stereotaxic Apparatus

Histopathological Analysis

Following induction, brain tissues from experimental and control groups were collected for histopathological evaluation. The tissues were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) to examine structural changes, demyelination, inflammatory cell infiltration, and neuronal degeneration under a light microscope. Photomicrographs were captured at multiple magnifications to document pathological features and to compare lesion severity between treated and control animals.

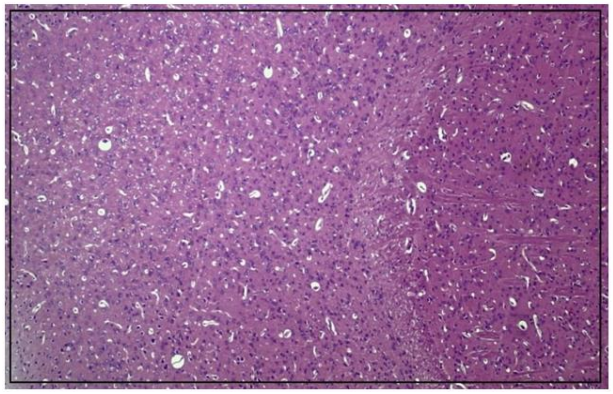

Fig. 1: Normal control: Mice brain tissue (X50)

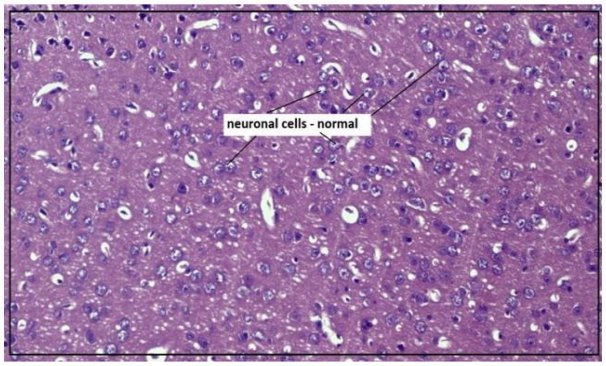

Fig. 2: Normal control: mice brain Cortical region showing Glial cells & neuronal cells normal morphology – NAD+ (X100)

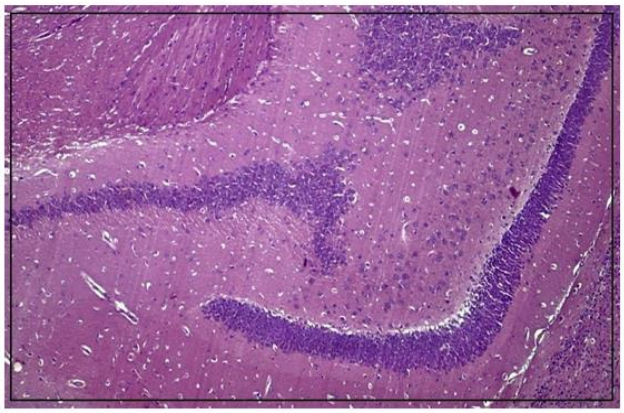

Fig. 3: Normal Control, Mice Brain: hippocampus region showing normal morphology (X50)

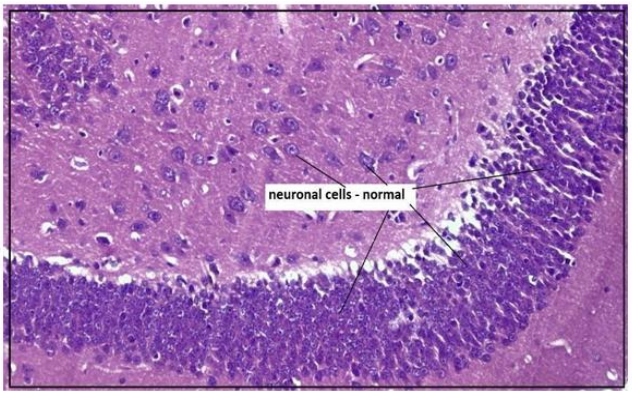

Fig. 4: Normal Control, Mice Brain: hippocampus region showing normal morphology – NAD+ (X100)

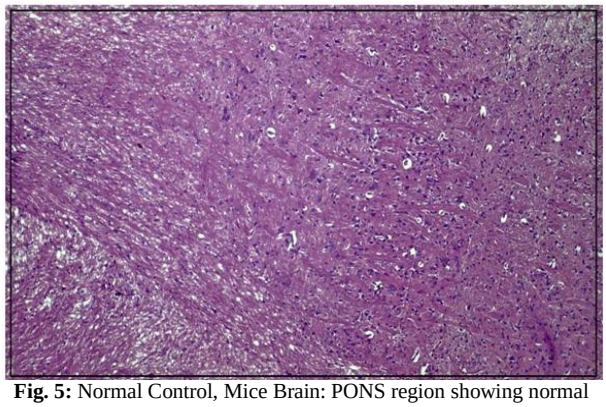

Fig. 5: Normal Control, Mice Brain: PONS region showing normal morphology (X50)

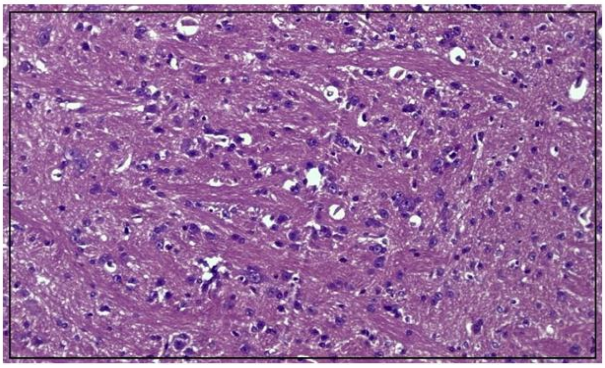

Fig. 6: Normal Control, Mice Brain: PONS region showing normal morphology – NAD+ (X100)

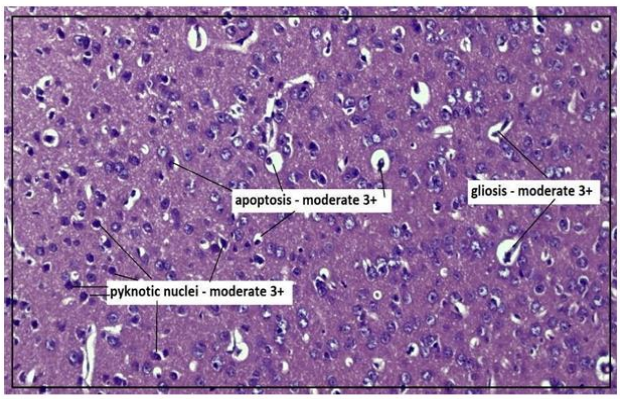

Fig.7: Positive Control, Mice Brain: Cortical region showing Pyknotic nuclei with Gliosis, apoptosis - Moderate: 3+ (X100)

Fig. 8: Positive Control, Mice Brain: PONS area showing congestion with Gliosis and inflammation were Severe – 4+ (X50)

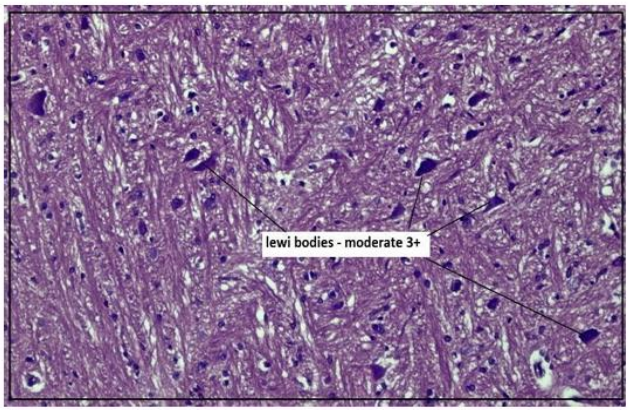

Fig. 9: Positive Control, Mice Brain: PONS area showing Lewi bodies were Moderate – 3+ (X100)

Interpretation of Histopathological Studies

In the present study, histopathological evaluation of the mouse brain revealed the following observations:

- **Normal Control Group:**
  Brain sections from normal control mice exhibited intact architecture, with glial and neuronal cells showing normal morphology and no detectable abnormalities (NAD).
- **Positive Control Group:**
  - Cortical and Hippocampal Regions: Moderate (3+) evidence of pyknotic nuclei accompanied by gliosis and apoptotic changes.
  - Pons Region: Severe (4+) vascular congestion with marked gliosis and inflammatory infiltration.
  - Additional findings included moderate (3+) neuronal hyperplasia and the presence of moderate (3+) Lewy body–like inclusions.

These findings confirm the successful induction of neuroinflammatory and neurodegenerative changes consistent with multiple sclerosis–like pathology in the positive control animals.

Objective 2 Development and Validation of Wistar Albino Rat Model for Multiple Sclerosis Using a Combination of Ethidium Bromide and Copper-Based Agents

Histopathological Studies

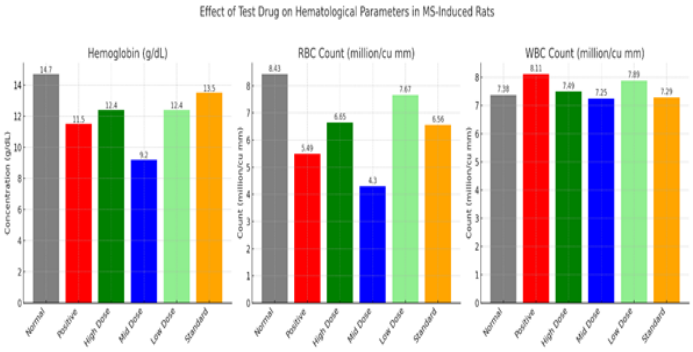

Fig.10: Hematological Parameters

Histopathological examination of brain tissues from Wistar albino rats was carried out to validate the induction of multiple sclerosis–like pathology. The harvested brain samples were fixed in 10% buffered formalin, processed, and embedded in paraffin. Thin sections (5 µm) were prepared and stained with hematoxylin and eosin (H&E) to evaluate cellular and structural alterations. Microscopic analysis focused on identifying hallmarks of demyelination and neuroinflammation, including pyknotic nuclei, gliosis, neuronal degeneration, vascular congestion, and inflammatory cell infiltration across cortical, hippocampal, and pontine regions. Photomicrographs were captured at appropriate magnifications to document lesion severity and to compare induced groups with controls, thereby confirming the establishment of the MS rat model.

Discussion

The hematological results indicate that multiple sclerosis induction with ethidium bromide and copper sulphate led to significant deviations from normal values, particularly reduced hemoglobin concentration and red blood cell count, alongside elevated mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). These changes are consistent with neuroinflammation-associated hematological alterations often observed in MS models. Treatment with the test drug demonstrated dose-dependent improvements across most parameters. The high-dose group showed notable recovery in hemoglobin levels and RBC count, comparable to the standard-treated group. Platelet counts, which were elevated in the positive control group, were moderated by test drug administration, suggesting a potential role in regulating inflammatory or coagulation-related changes. Overall, these findings support the ameliorative potential of the test drug in restoring hematological balance in MS-induced rats, thereby complementing its anticipated neuroprotective effects.

Conclusion

This study successfully established a reproducible method for MS induction in transgenic rodent models and highlighted the ameliorative efficacy of fenofibric acid as a test formulation. The combined evidence from histopathology, hematology, behavioral studies, and computational docking strongly supports its neuroprotective, anti-inflammatory, and immunomodulatory potential. The findings provide a scientific basis for fenofibric acid as a candidate therapeutic agent for MS and warrant further investigation, including long-term preclinical studies and eventual clinical translation. Moreover, the study emphasizes the value of integrating transgenic models with phytochemical-based interventions to develop safer, cost-effective alternatives to current disease-modifying therapies.

Conflict of Interests

The authors declare no conflict of interest

Funding: This study received no specific funding from public, commercial, or not for profit funding agencies.

AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Data Availability

Data will be available on request

References

[1] Aktas, O., Kieseier, B., Hartung, H. P., & Zipp, F. Neuroprotection, regeneration and immunomodulation: Broadening the therapeutic repertoire in multiple sclerosis. *Trends in Neurosciences. 2010;33*(3), 140–152. https://doi.org/10.1016/j.tins.2009.12.005

[2] Al-Izki, S., Pryce, G., Jackson, S. J., Giovannoni, G., Baker, D., & Amor, S. (2011). Practical guide to experimental autoimmune encephalomyelitis in the mouse. *Practical Neurology, 11*(1), 25–34. https://doi.org/10.1136/jnnp.2010.211714

[3] Alirezaei, Z., & Pourhanifeh, M. H. (2019). The therapeutic potential of flavonoids in multiple sclerosis. *Journal of Neuroimmunology, 329*, 16–23. https://doi.org/10.1016/j.jneuroim.2018.08.007

[4] Ammitzbøll, C., Søndergaard, H. B., Aljabri, A., & Sellebjerg, F. (2019). Natural killer cells in multiple sclerosis: The yin and yang of disease pathogenesis and regulation. *Brain, 142*(1), 23–35. https://doi.org/10.1093/brain/awy290

[5] Baker, D., & Amor, S. (2014). Experimental autoimmune encephalomyelitis is a good model of multiple sclerosis if used wisely. *Multiple Sclerosis and Related Disorders, 3*(5), 555–564. https://doi.org/10.1016/j.msard.2014.05.002

[6] Behan, P. O., & Chaudhuri, A. (2010). Toxic demyelination: Experimental models and relevance to multiple sclerosis. *Current Opinion in Neurology, 23*(3), 233–239. https://doi.org/10.1097/WCO.0b013e328338f5f7

[7] Benveniste, E. N., & Qin, H. (2018). Type I interferons as anti-inflammatory mediators. *Science Immunology, 3*(24), eaau6291. https://doi.org/10.1126/sciimmunol.aau6291

[8] Bjelobaba, I., Begovic-Kupresanin, V., Pekovic, S., & Lavrnja, I. (2017). Animal models of multiple sclerosis: Focus on experimental autoimmune encephalomyelitis. *Journal of Neuroscience Research, 96*(6), 1021–1042. https://doi.org/10.1002/jnr.24131

[9] Braidy, N., Behzad, S., Habtemariam, S., & Chi, Y. (2017). Neuroprotective effects of polyphenols in multiple sclerosis. *NeuroMolecular Medicine, 19*(3), 1–16. https://doi.org/10.1007/s12017-017-8452-2

[10] Constantinescu, C. S., Farooqi, N., O'Brien, K., & Gran, B. (2011). Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *British Journal of Pharmacology, 164*(4), 1079–1106. https://doi.org/10.1111/j.1476-5381.2011.01302.x

[11] Correale, J., & Farez, M. F. (2015). The role of astrocytes in multiple sclerosis progression. *Frontiers in Neurology, 6*, 180. https://doi.org/10.3389/fneur.2015.00180

[12] Denic, A., Johnson, A. J., Bieber, A. J., Warrington, A. E., Rodriguez, M., & Pirko, I. (2011). The relevance of animal models in multiple sclerosis research. *Pathophysiology, 18*(1), 21–29. https://doi.org/10.1016/j.pathophys.2010.04.004

[13] Dendrou, C. A., Fugger, L., & Friese, M. A. (2015). Immunopathology of multiple sclerosis. *Nature Reviews Immunology, 15*(9), 545–558. https://doi.org/10.1038/nri3871

[14] Ebrahimi-Kalan, A., & Salehi, B. (2020). Herbal medicines for multiple sclerosis: A review. *Phytotherapy Research, 34*(7), 1550–1574. https://doi.org/10.1002/ptr.6637

[15] Esposito, S., & Cuzzocrea, S. (2010). Antioxidant therapy in multiple sclerosis. *Free Radical Biology and Medicine, 52*(6), 1484–1499. https://doi.org/10.1016/j.freeradbiomed.2010.02.001

[16] Farez, M. F., & Correale, J. (2016). Regulation of oligodendrocyte survival and CNS remyelination by immune cells. *Neuroimmunology&Neuroinflammation 3*(2), 74–82. https://doi.org/10.4103/2347-8659.176574

[17] GBD 2021 Multiple Sclerosis Collaborators. (2022). Global, regional, and national burden of multiple sclerosis, 1990–2019. *The Lancet Neurology, 21*(7), 585–601. https://doi.org/10.1016/S1474-4422(22)00142-0

[18] BV Ramana, VV Kumar, PNR Krishna, CS Kumar, PUM Reddy, TN Raju," Effect Of Quercetin On Galactose-Induced Hyperglycaemic Oxidative Stress In Hepatic And Neuronal Tissues Of Wistar Rats" Acta Diabetologica. 2006;43:135-141.

[19] Kishore Babu.M, Ramana B.V and Murthy T.E.G.K, "Influence of polymers on release rate of carvedilol from transdermal films", Indian Drugs, 2008; 45 (12):970-974

[20] Durg S, Bavage S, Satrasala N, Charan CS. Efficacy and Safety of Withania somnifera (Indian Ginseng) in Rheumatoid Arthritis: A Systematic Review of Evidence from Experimental Studies to Clinical Trials. Ashwagandha. 2024:333-53.

[21] Neelima S, Pradeep Kumar M, Bageswari B, Sainath Reddy C, Sai Suresh G, Haripriya M, Grace A. Investigation of in vitro anti-inflammatory activity of Oxalis latifolia Kunth whole plant. Future J Pharm Health Sci. 2024;4(4):1–7.

[22] Ghasemi, N., Razavi, S., & Nikzad, E. (2017). Multiple sclerosis: Pathogenesis, symptoms, diagnoses, and cell-based therapy. *Cell Journal, 19*(1), 1–10. https://doi.org/10.22074/cellj.2017.4862

[23] Gutiérrez, M. R., Romero-Sánchez, C., & Angulo, S. (2018). Polyphenols as neuroprotective agents in multiple sclerosis. *Nutrients, 10*(10), 1408. https://doi.org/10.3390/nu10101408

[24] Hauser, S. L., & Cree, B. A. C. (2020). Treatment of multiple sclerosis: A review. *American Journal of Medicine, 133*(12), 1380–1390. https://doi.org/10.1016/j.amjmed.2020.05.049

[25] Hemmer, B., Kerschensteiner, M., & Korn, T. (2015). Role of the immune system in multiple sclerosis. *Nature Reviews Neuroscience, 16*(12), 347–360. https://doi.org/10.1038/nrn3902